Original Article

pISSN, eISSN 0125-5614
M Dent J 2017; 37 (3) : 301-310

Microleakage Of Class II Bulk-fill And Conventional Resin Composite Restorations With And Without Enamel Preservation At Gingival Wall

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Objectives: This *in vitro* study tested the hypothesis that preserving a thin enamel layer at the gingival margin and using bulk-fill resin composites could minimize microleakage of class II resin composite.
Materials and Methods: Thirty-six human third molars were randomly divided into three groups of 12 specimens each: Filtek Bulk Fill Posterior Restorative in Capsules (BFC), Filtek Bulk Fill Posterior Restorative in Syringes (BFS) and Filtek Z350 XT (Z350). Teeth were prepared on two sides for a class II cavity (3 mm buccolingually x 2 mm mesiodistally at occlusal and 1.5 mm at coronal x 4 mm of axial depth) with 0.5 mm under the CEJ on one side (NP) and 0.5x1 mm of thin enamel at the gingival margin was preserved on the other side (EP). The teeth were then restored, thermocycled, immersed in 0.5% methylene blue solution for 24 hours and sectioned mesiodistally through the restorations. Dye penetration was evaluated at the gingival margin by three blinded examiners using a 0-4 ordinal scale. The Kruskal-Wallis test and Dunn test were used to compare differences in microleakage scores among the three restorative materials. Mann-Whitney U test was utilized to analyze the difference between enamel preserved (EP) and non-enamel preserved sides (NP) in the same restorative material. Tests were performed with the level of significance at $\alpha = 0.05$.
Results: Mann-Whitney U test showed that the "NP" groups had significantly higher microleakage score than the "EP" groups. The Kruskal-Wallis test revealed significant differences in microleakage scores among the three restorative materials ($P < 0.05$). Compared to "Z350", the "EP" group, "BFC" and "BFS" had significantly less microleakage score ($P = 0.001$) ($P = 0.028$). The "NE" group, "BFC" had significantly less microleakage score than "Z350" ($P = 0.001$).

Keywords: Bulk-fill resin composite, Class II resin composite, Enamel preserved, Gingival margin, Microleakage, Thin enamel layer

How to cite: Pisuttiwong O, Leevailoj C. Microleakage of class II bulk-fill and conventional resin composite restorations with and without enamel preservation at gingival wall. M Dent J 2017; 37: 301-310.

Introduction

Tooth-colored filling material, resin composites are now widely used for posterior teeth restoration because of their ability to mimic the color of natural teeth and meet patient's demand in esthetic appearance. Moreover, restoration using resin composite can be completed in one visit, making it convenient for both the dentist and the patient. Several clinical studies have reported the long-term durability of resin composite.[1-3] However, some clinical problems concerning restoring tooth structure with resin composite still remains such as microleakage at the gingival wall of class II cavity resin composite restoration [4], which might lead to post-operative hypersensitivity, secondary caries and pulpal pathology.[4-6]

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Received : 25 July 2017 Accepted : 16 October 2017

Microleakage was defined as the clinically undetectable passage of bacteria, fluid, molecules, or ions between a cavity wall and the restorative materials.[7] There were multiple factors that cause microleakage when restoring teeth with resin composite. One of the main causes was polymerization shrinkage of the material.[4] Other factors included the cavity configuration factor, coefficient of thermal expansion, adhesive bond strength, hygroscopic expansion and modulus of elasticity of the restorative materials.

Ideally, for resin composite restoration enamel preservation should be maximized for better adhesive properties. Bonding to enamel provided a better bond in comparison to dentin or cementum.[4] Leevairoj C. et al. found that microleakage at the gingival level of class II cavities restored with resin composite was higher than at the occlusal level.[8] When proximal dental caries penetrated into dentin, the dental substrate was extensively damaged. It might penetrate down under the CEJ, leaving a thin layer of enamel at the gingival margin undamaged. This unsupported enamel was normally removed for two reasons; Firstly, The arrangement of enamel rods at the CEJ area was irregular and lacking definite form which might have an affect on bonding efficiency.[9] Secondly, the unsupported enamel might be fractured as a consequence of polymerization shrinkage stress.[10] In addition to make a straight horizontal gingival wall, the dentist might decide to grind this fine undamaged enamel out.

Current resin composites have good physical properties of hardness, flexural strength, and fracture toughness, as well as low shrinkage and low wear. However, because of the low depth of cure, conventional resin composites required the addition of multiple separate cured layers. This was called “Incremental placement” and was time consuming. One advantage of bulk-fill resin composites was that the dentist can restore thicker layers of material compared to conventional resin composite and allow complete polymerization to take place.[11] The placement of large increments of bulk-fill resin composite into a cavity increased the potential of creating high shrinkage stress. However, a study has shown that the mean values of polymerization stress for most of the bulk-fill products were not statistically different compared to conventional resin composites.[12] Filtek Bulk Fill Posterior Restorative in capsule and syringe type was launched onto the market with the same composition but a different application method. The key manufacturing features relate to improved polymerization shrinkage with a greater depth of cure. Testing the microleakage of this bulk-fill product in both capsule and syringe type is, therefore, of interest.

No current research has investigated the microleakage from cavities where a thin enamel layer was left at the gingival wall. Therefore, this study examined the effect of preserving a thin enamel layer at the gingival wall on the microleakage of class II resin composite restoration. In addition, the microleakage was compared between bulk-fill and conventional resin composites.

Methods and Materials

Materials used are shown in Table 1. Thirty-six non-carious, non-restored, uncracked extracted human third molars were stored in 0.5% thymol solution at 4 °C until required for use. The teeth were randomly divided into three groups of 12 teeth each. Seventy-two standardized class II cavities occlusal to the CEJ at 1 mm were made in both proximal sides of each molar using a cylinder diamond bur diameter 1.5 mm (DIA TESSIN, BKK, Thailand). Each cavity measured 3 mm wide buccolingually, 2 mm in mesiodistal at occlusal 1/3 and 1.5 mm at coronal1/3. Cutting tip edge diamond burs of diameter 1 mm (Cross Tech, BKK, Thailand) were then used to deepen the cavity inferior to CEJ 0.5 mm, leaving 0.5 mm axial depth and 1 mm height of thin enamel at the gingival margin in both proximal sides. Mesial or distal sides of each molar were randomly selected to preserve the thin enamel on one side (EP). For the opposite side, the thin enamel was eliminated to create a straight horizontal gingival wall (NP). All teeth were flattened parallel to the occlusal surface at 3.5 mm height from the CEJ with a carborundum disc (Miltex, Rietheim-Weilheim, Baden-Württemberg, Germany) (Figure 1).

Table 1 Materials used with the manufacturer's information, composition and lot numbers

Material / Manufacturer	Composition	Lot #
Filtek Bulk Fill Posterior Restorative (capsule type) 3M ESPE, St. Paul, MN, USA	AUDMA, DDDMA, UDMA, Silica (20 nm non-agglomerated/aggregated), Zirconia (4-11 nm non=agglomerated/aggregated), Zirconia/Silica aggregated cluster (20 nm silica combined with 4-11 nm zirconia), Ytterbium trifluoride (100 nm aggregated)	N666574
Filtek Bulk Fill Posterior Restorative (syringe type) 3M ESPE, St. Paul, MN, USA	AUDMA, DDDMA, UDMA, Silica (20 nm non-agglomerated/aggregated), Zirconia (4-11 nm non=agglomerated/aggregated), Zirconia/Silica aggregated cluster (20 nm silica combined with 4-11 nm zirconia), Ytterbium trifluoride (100 nm aggregated)	N611596
Filtek Z350 XT 3M ESPE, St. Paul, MN, USA	UDMA, BIS-EMA, PEGDMA, Silica (20 nm non-agglomerated/aggregated), Zirconia (4-11 nm non=agglomerated/aggregated), Zirconia/Silica aggregated cluster (20 nm silica combined with 4-11 nm zirconia)	N652159
Adper Scotchbond Multi-Purpose Adhesive 3M ESPE, St. Paul, MN, USA	Etchant: 35% Phosphoric acid Primer: HEMA, Polyalkenoic acid copolymer Adhesive: Bis-GMA, HEMA	N616851

Abbreviations: AUDMA, AROMATIC URETHANE DIMETHACRYLATE; DDDMA, 1,12-DODECANE DIMETHYCRYLATE; UDMA, DIURETHANE DIMETHACRYLATE; BIS-EMA, BISPHENOL A ETHOXYLATE DIMETHACRYLATE; PEGDMA, POLYETHYLENE GLYCOL DIMETHACRYLATE; BISGMA, BISPHENOL A GLYCIDYL METHACRYLATE; HEMA, 2-HYDROXYETHYL METHACRYLATE

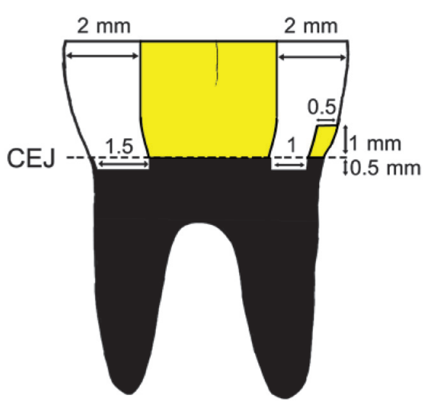

Fig 1 Dimensions of class II cavity preparation composed of non-enamel preserved side (left) and enamel preserved side (right)

In restorative procedure, the cavitated teeth were placed adjacent to the molar tooth in a clay block to replicate the clinical situation. Automatrix (Kerr, Orange, CA, USA) was used with a transparent band (5.0 mm). Half of each experimental group (6 specimens) was randomly restored the “EP” side prior to “NP” side. Each first restored side was wrapped with thin aluminum foil before the second side was restored. The cavity surface was conditioned using Adper Scotchbond Multi-Purpose Adhesive (3M ESPE, St. Paul, MN, USA). The process was performed following the manufacturer’s instructions as follows: etch with 32% phosphoric acid for 15 seconds, rinse with water from triple syringe for 15 seconds, blot dry with triple syringe with air density at 2 bar pressure for 5 seconds, apply primer with microbrush 2 times for 5 seconds each, completely dry with air density at 2 bar pressure for 10 seconds, apply bonding with microbrush for 5 seconds, then light cure for 10 seconds. The position of the LED light-curing tip (DEMI PLUS, Kerr, WI, USA) was adjusted perpendicular and close to the occlusal surface of the cavity. Periodic Level Shifting (PLS) mode which is shifting of the output intensity from 1100 mW/cm^2 to a peak of 1330 mW/cm^2 in a short time for multiple times throughout the curing cycle was used. The light-curing unit was recharged every day before use. Blade no.12 (Swann-Morton, Shieffield, Eng) was used to finish the restoration’s margin. All preparation and restoration were performed by one operator under dental loupes at magnifications of 2.8X (Orascoptic, Middleton, WI, USA).

Group 1 (12 specimens); Filtek Bulk Fill Posterior Restorative, capsule type (BFC) (shadeA2, 3M ESPE, St. Paul, MN, USA) was placed 4 mm in one time to completely fill the cavity using a composite dispenser gun.

Group 2 (12 specimens); Filtek Bulk Fill Posterior Restorative, syringe type (BFS) (shadeA2, 3M ESPE, St. Paul, MN, USA) was placed 4 mm in one time to completely fill the cavity using a W3 Composite Instrument (Hu-Friedy, Chicaco, IL, USA). For both Group 1 and Group 2, resin composites were condensed with a 5A XTS Plugger (Hu-Friedy, Chicaco, IL, USA) in10 times. Then, the materials were light-cured at occlusal, buccal and lingual sides for 20 seconds on each side.

Group 3 (12 specimens); Filtek Z350 XT (Z350) (shadeA2, 3M ESPE, St. Paul, MN, USA) was placed into the cavity in two layers using a W3 Composite Instrument (Hu-Friedy, Chicaco, IL, USA). The first 2 mm layer was plugged with a 5A XTS Plugger (Hu-Friedy, Chicaco, IL, USA) in 10 times and then light-cured on the occlusal side for 20 seconds. The next horizontal incremental layer was performed as the first layer and light-cured at occlusal, buccal and lingual sides for 20 seconds on each side.

Evaluation for microleakage

All restored specimens were thermocycled (Certiga, Unterhaching, Austria) between 5 °C and 55 °C for 5,000 cycles with 30-second dwell time to simulate clinical aging after 24 hours storage in distilled water at 37 ± 2 °C. The root tips were coated and sealed with flowable resin composite (Premise, Kerr, Orange, CA, USA). Crown and root were double coated with red nail polish, leaving only a 1 mm gingival margin of restoration. All specimens were dried for 24 hours and then immersed in 0.5% methylene blue solution for 24 hours. After removing the nail polish and rinsing with water for 5 minutes, the teeth were placed into an acrylic block with the occlusal surface parallel to the ground position and sectioned mesiodistally through the restorations using a low speed cutting machine (model ISOMET 1000, Buehler, Binghamton, NY, USA). The sectioned specimens, both buccal and lingual side, were examined at 20X magnification using a stereomicroscope (ML 9300 MEIJI TECHNO, Saitama, Japan) and standardized digital images were obtained. The images were randomly arranged with Keynote program to evaluate dye penetration at the gingival margin individually by three blinded examiners who were restorative dentists. All examiners were calibrated and had excellent strength of reliability in ICC (intraclass correlation coefficient interpretation). Consensus was forced in case of disagreement occurred after the evaluation all of specimens by selecting the issue images to rediscuss the score.

All data were analyzed with statistical software (SPSS 22.0; spss). All test were performed with the level of significance at $\alpha = 0.05$. Due to the nature of microleakage score as ordinal scale, non-parametric test was utilized.

1. Kruskal-Wallis test were utilized to analyze whether there is any significant differences between 3 restorative materials, both in enamel preserved (EP) and non-enamel preserve (NP) groups. After the result showed statistical significant difference ($P < 0.05$), multiple comparison test (Dunn test) was performed to determine which pair of techniques is different.

2. Mann-Whitney u test was utilized to analyze the difference between two groups, “EP” and “NP” in the same restorative material.

Results

The number of specimens available for evaluation was 141 from 144 specimens. Three fillings were lost during the cutting procedure. The dye penetration and mode of scores at the gingival wall of Class II resin composite restorations are shown in Table 2.

Results of Mann-Whitney U test in Table 2 showed that the “NP” group had significantly higher microleakage scores than the “EP” group for all of the three restorative materials.

Table 3 revealed that the “EP” group, “Z350” showed statistically significant higher microleakage scores than “BFC” (P = 0.001) and “BFS” (P = 0.028). For the “NP” group, “Z350” showed statistically significant higher microleakage scores than “BFC” (P = 0.001) but no significant difference with “BFS”. “BFC” and “BFS” showed no significant difference in microleakage score between each other.

Table 2 Distribution of the microleakage score, Mode and Mann-Whitney U test between enamel preserved groups and non-enamel preserved groups of the three restorative materials.

Group		Microleakage score					Total	Mode of score	Asymp. Sig. (P-value)
		0	1	2	3	4			
BFC	EP	20	3	-	-	-	23	0	0.003*
	NP	11	5	1	1	5	23	0	
BFS	EP	19	-	-	-	5	24	0	0.003*
	NP	6	3	4	2	9	24	4	
Z350	EP	9	8	-	2	5	24	0	0.001*
	NP	2	2	1	3	15	23	4	

Upper case asterisk indicate statistical significant difference (p ≤ 0.05) Abbreviations: BFC, Filtek Bulk Fill Posterior Restorative (capsule type); BFS, Filtek Bulk Fill Posterior Restorative (syringe type); Z350, Filtek Z350 XT; EP, Enamel preserved; NP, Non-enamel preserved

Table 3 Multiple comparison (Dunn test) between the three restorative materials of enamel preserved groups and non-enamel preserved groups

Group	Asymp. Sig. EP (*P*-value)	Asymp. Sig. NP (*P*-value)
BFC versus BFS	0.976	0.247
BFC versus Z350	0.001*	0.001*
BFS versus Z350	0.028*	0.170

Upper case asterisk indicate statistical significant difference (p ≤ 0.05) Abbreviations: BFC, Filtek Bulk Fill Posterior Restorative (capsule type); BFS, Filtek Bulk Fill Posterior Restorative (syringe type); Z350, Filtek Z350 XT; EP, Enamel preserved; NP, Non-enamel preserved

Discussion

A microleakage test is one of the methods used to measure the quality of resin composite restoration. Many researchers used this test to measure the properties of dental restorations because of its simplicity in performing the test. The data obtained could be easily evaluated and samples were not destroyed during measurement interpretation.[15] However, there were also disadvantages to the microleakage test, the evaluation of microleakage largely depends on the observer's interpretation and the microleakages are scored from 2D image, while the restoration material is shaped in 3D. Therefore, the microleakage results might have some discrepancies from actuality.[16] According to the ISO/TS11405 (2015), many tracer solutions have been used for microleakage test. It is obviously impractical to use a dye particle which has a diameter greater than the internal diameter of the dentinal tubules (1-4 m).[17] The recommended size of dye particle is a diameter equal to the bacterial size or smaller at around 2 µm. Considering the penetration capacity of methylene blue, its use is considered as a good tracer for microleakage test because the area of methylene blue is very small (0.52 nm^2) when compared to the mean size of a bacteria[16] and its penetration into the specimen can be easily detected by stereomicroscope. However, if the detection of a very severe nanoleakage test was required, such as analyzing of discrepancy between the depth of the demineralized zone and monomer diffusion, silver nitrate would be a better choice because of the diameter of the silver ion (0.059 nm) and its strong optical contrast of silver paticles.[18] Previous concentrations of methylene blue used ranged from 0.5% to 10%, while time of immersion of specimens in the dye ranged between 4 and 72 hours or more.[17] None of the concentrations are ideal but the recommended immersion time from the ISO/TS11405 (2015) is 24 hours. In this study, methylene blue at 0.5% and 24 hours immersion time was used because of its quality being high enough for testing microleakage, ease of preparation and cost effectiveness.

In this research, two bulk-fill products ("BFC" and "BFS") were compared with conventional resin composite ("Z350"). The products all came from the same company and contained the same type of filler in nanometric scale. "Z350" is well known and widely used in dental clinics. The manufacturer claims that "BFC" and "BFS" have 4 mm depth of cure with less polymerization shrinkage. This concurred with the results in Table 2, indicating that the majority of the specimens in "BFC" and "BFS" showed no microleakage (score 0), while less than half of "Z350" specimens showed no microleakage (score 0) or mild microleakage (score 1). The majority of “Z350” specimens showed severe microleakage (score 4).

Neither of two bulk-fill products represented others bulk-fill products in the market due to difference in compositions and properties.[11,19,20] It is known that the shear stresses induced by injection technique can improved marginal adaptation instead of a hand instrument.[21] Resin composite was placed into the prepared cavity by a hand instrument in “BFS” group, similarly to the conventional resin composite; while, “BFC” resin composite was dispensed through a capsule tip by a composite dispenser gun at the deepest part of prepared cavity, and then the tip was slowly withdrawn as the cavity was filled. Hence, “BFC” should perform better microleakage score than “BFS”. Nevertheless, the results showed no significant difference in microleakage score between using “BFC” and “BFS”.

One thing concerning the use of “BFC” is the diameter of the tip being 2 mm. Therefore, in small cavities with width less than 2 mm the tip may not reach till the cavity depth, and this can result in poor adaptation of restorative resin if the force to compress the thick layer of resin composite is not high enough. In this research, “BFC” still showed good results for microleakage at a gingival margin of 1.5 mm. This might be because the cavity design size at the occlusal approached 2 mm and the tip could be pushed down into the cavity.

Focusing on dental substrates, microleakage scores ranged from no leakage (0) to the highest severe leakage (4). The samples were divided into three parts by an imaginary line in the Keynote program (Illustration 2). In the first part (score 1), there was a difference in the distance of dye penetration because the height of the enamel in “EP” groups, making the leakage pathway to reach the second part longer than in “NP” groups. Results of microleakage distribution in Table 2 showed the scores of “EP” group were mostly 0-1 (no to mild microleakage) (Illustration 3a). In contrast, the majority of microleakage scores for “NP” group were 3-4 (moderate to severe microleakage) (Illustration 3b). These findings concurred with other authors who reported that leakage mostly occurred at the dentin surface.[22-24] The preservation of a thin enamel layer at the gingival wall in this research increased the leakage distance from the outside margin to the dentin. The thin enamel layer (0.5 mm) was still preserved, even without the supporting dentin due to its location being at the proximal, which is not directly subjected to occlusal stress. However, this enamel layer might become fractured as a consequence of polymerization shrinkage stress [10] Therefore, Future research might test for microleakage combined with mechanical loading to observe how occlusal force impacts on this thin enamel layer. In this study, etch and rinse system was used which considered as a gold standard adhesive. A study has shown that different adhesive systems had an affect on microleakage scores in enamel substrate but not in dentin substrate.[25] Therefore, the results of this study may be different if other adhesive systems was used.

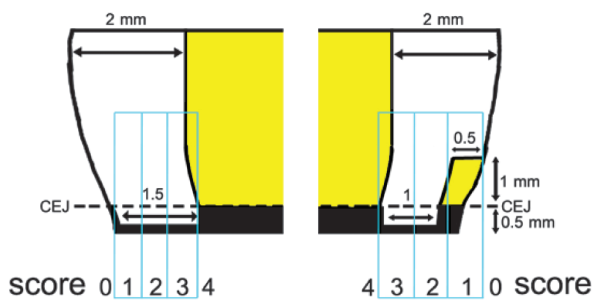

Fig 2 Scoring of microleakage

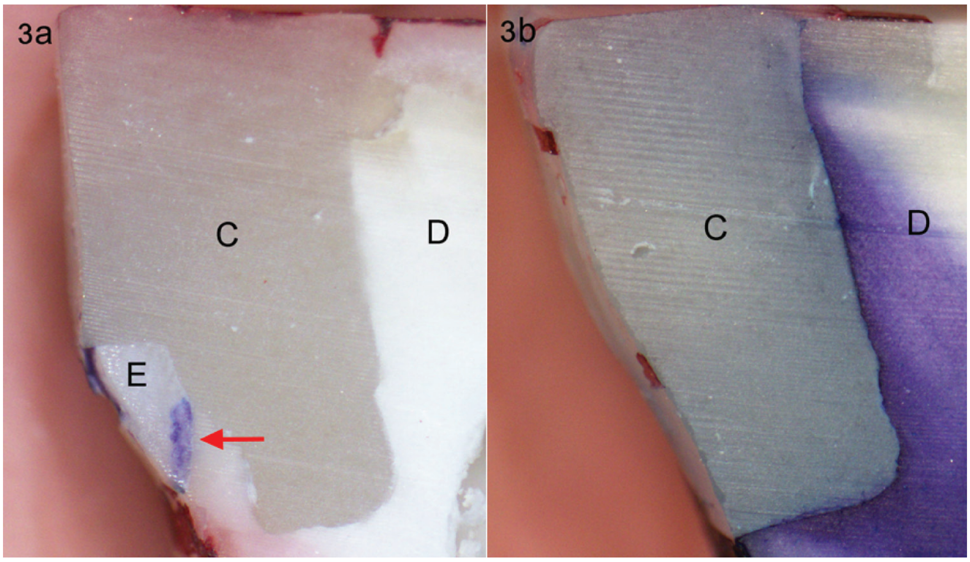

Fig 3 Representative specimen 3a showing microleakage of the enamel preserved side of Filtek Bulk Fill (capsules) (score 0), the red arrow pointing to the dye that penatration into enamel surface. Representative specimen 3b showing microleakage of the non-enamel preserved side of Filtek Z350 XT (score 4). (E: enamel; D: dentine; C: resin composite)

Dye penetration into other areas, not at the dental-restorative junction, was found in some specimens (25 pieces from 141 pieces), mostly occurred in "EP" groups (Illustration 3a), which did not affact the interpreting of microleakage score. Reasons for dye penetration in dental substrate beyond the dental-restorative junction were not determined, but may be due to microcrack or cracks that could be created from the preparation procedure. The higer C-factor have also been reported to produce higher contraction stresses [26], especially in "EP" groups in which ratio of the bonded surface area to the free surface area of cavity is higher than "NP" groups, stresses might created microcrack in the thin layer of preserved enamel.

Thermocycling was a widely accepted method for *in vitro* microleakage studies.[27] A literature review concluded that 10,000 cycles corresponded approximately to 1 year of *in vivo* functioning.[28] The ISO/TS11405 (2015) suggests that a thermocycling regimen comprising of 500 cycles in water between 5 °C and 55 °C with at least 20 seconds dwell time is an appropriate artificial aging test. Here, 5,000 test cycles were used as an aging technique to simulate the intraoral temperature. Further research might evaluate results for 10,000 cycles to replicate 1 year of *in vivo* functioning and observe how the added cycles affect on microleakage of all experimental groups.

Regarding clinical implications, preserving the enamel at the gingival margin would made it easier for the dentist to build up contact or prevent moisture from sulcular fluid due to the higher margin of restorations compared to cavities without preserving. Furthermore, it would be easier for patients to perform routine cleaning when the margin of the restorative materials was not under the gingiva. Limited studies have investigated the thin enamel and further research is necessary to determine any possible disadvantages of preserving this thin enamel layer.

In Conclusions, under controlled condition of this research, microleakage of class II resin composite filling occurred in all the three experimental materials "BFC", "BFS" and "Z350" for both "EP" and "NP" groups. However, preserving thin layer of enamel ("EP") and use of two bulk-fill products ("BFC" and "BFS") reduced microleakage.

Funding, competing interest, ethical approval

Funding: None

Competing interest: None declared

Ethical approval: The Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (Ethic code: HREC-DCU-P 2015-002)

Acknowlegement

This research would not have been possible without the ongoing support and guidance of the following people, for which I would like to take this opportunity to thanks.

I would like to express my sincere appreciation to my program director and advisor; Assoc. Prof. Chalermpol Leevailoj for sharing his expertise and knowledge, his love and passion for research which had greatly motivated me to focus on my work, and provided me with the experience of high quality research and its required attitudes. His understanding, advice and support throughout my whole postgraduate course are greatly appreciated.

My gratitude is also extended to Assoc. Prof. Chanchai Hosawaun for statistic consulting, whose statistical methods were extraordinarily helpful.

All of this would have been unachievable without the endless support, caring and understanding from them.

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